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Effect of Plastocyanin and Phycocyanin on the Photosensitivity of Chlorophyll-Containing Bilayer Membranes

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Summary. Photovoltaic effects were studied in bilayer membranes constructed from phosphatidyl choline, monogalactosyl diglyceride, chlorophyll *a* and β-carotene. It was demonstrated that the biliprotein C-phycocyanin enhanced the photosensitivity of these membranes. Plastocyanin, an important photosynthetic electron transfer protein, was also found to be effective in enhancing the membrane photovoltage. The C-phycocyanin and plastocyanin were effective on opposite sides of the membrane. Plastocyanin operates to transfer electrons into the membrane, while C-phycocyanin directs electron transfer from the membrane. Membranes containing monogalactosyl diglyceride were found to be extremely stable and were most susceptible to enhancement of photosensitivity by introduction of the proteins. The plastocyanin and C-phycocyanin when used together appeared to operate synergistically.

Photovoltaic effects of pigmented bilayer membranes have been studied intensively in recent years and a large number of the contributions from several laboratories have been reviewed [3, 11, 22]. Such membranes containing the appropriate pigments are photosensitive if a gradient in redox potential, pH or electrical potential is created across the membrane.

Studies of membranes containing photosynthetic pigments such as chlorophyll and β-carotene [7, 20, 23, 24], chlorophyll and xanthophyll [10, 21] and chloroplast extract from spinach [12, 14, 25] generated a great deal of interest because of their potential as model systems for investigation of photosynthesis and solar energy conversion [5]. There are rather well-defined problems associated with these thoughts and they may be enumerated. (i) The analogy of the pigment BLM system to the photosynthetic system is at best premature. The BLM system does not, as currently studied in any laboratory, contain membrane proteins or membrane structure in any fashion as sophisticated as the thylakoid membrane. (ii) The thought that solar energy conversion is achievable

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with the pigmented BLM also requires close scrutiny. In the usual experimental situation [7, 10, 12, 20, 21, 23–25] the photovoltaic effect simply represents a dissipation of a pre-existing electrochemical gradient. Even this process has a low quantum yield (10^{-5} *vs.* about 0.5 for the photosynthetic process). (iii) The maximum stability of the BLM system as currently used is no more than a matter of hours.

It is important in embarking on additional studies of the pigmented BLM to confront these problems. The model membrane containing chlorophyll and other components present in the photosynthetic lamella cannot be viewed as a simple model system of direct relevance to the photosynthetic process or solar energy conversion. These model membranes do represent an unusual opportunity for the study of the interaction of well-defined systems under carefully controlled circumstances. This may in itself yield information which in the limit could lead to valuable insight into biological processes and at the very least help us to understand aspects of the photochemical behavior of some complex molecules.

Recent studies have been directed with limited success towards the problems enumerated. Initially a well-defined BLM system consisting of chlorophyll *a*, phosphatidyl choline, and β-carotene was demonstrated to be capable of producing photovoltages [20] similar to the less well-defined chloroplasts extract. Next a significant effort was made to elucidate possible specific photovoltaic effects with membranes containing in addition to chlorophyll *a* and β-carotene a single lipid of varying head group, hydrocarbon chain length, and degree of saturation of hydrocarbon chains [7]. No large enhancement of quantum efficiency was encountered.

Finally, the interaction of the biliproteins, accessory photosynthetic pigments, with the chloroplast extract BLM was demonstrated to enhance the quantum yield of the energy dissipative process by a modest factor of 2 to 3 and also to convert the energy dissipative process to that of energy storage [6]. The work with the biliproteins was extensive and included phycocyanin and phycoerythrin from blue-green and red algae as well as cryptomonads. A theoretical model has been suggested [15] to explain the mechanism of the energy dissipative process in terms of electron flow across the BLM and the electron-directing effect of the biliproteins which lead to energy storage. Modification of the membrane water interface is postulated as taking place when the biliprotein is introduced.

The present study extends the work with biliproteins to the well-defined chlorophyll *a*, β-carotene phospholipid BLM and also presents

experiments with the enhancement of photovoltaic processes with another biologically important molecule plastocyanin. The protein plastocyanin has been characterized as being an important agent in electron transfer from the cytochromes to P700 in Photosystem II [2]. The potential synergistic effect on quantum efficiency of biliprotein and plastocyanin is explored. Additional success with increasing the quantum efficiency of the photovoltaic effect in a significant manner is also reported for incorporation of monogalactosyl diglyceride in the phosphatidyl choline containing membranes. The presence of this lipid, which is an essential component of the photosynthetic lamella [1, 7], also increases the stability of the BLM by more than an order of magnitude.

Materials and Methods

Membranes were formed by applying 2 μl of membrane-forming solution with a gas-tight Hamilton microliter syringe, to a hole (1 mm in diameter) punched in a Teflon cup which was seated in a Lucite chamber [14]. The hole was always kept below the surfaces of the aqueous redox solutions inside and outside the cup and thus served as the point of communication between the solutions. The formation of the membrane and the thinning process was checked by means of an AC bridge (Wayne-Kerr B221 Universal Bridge), at 1 KC with a wave form generator (Wavetek, Multipurpose VCG Model 116), and an oscilloscope (Textronix 5103N with 5A22N differential amplifier and 5B10N time base amplifier). As soon as a drop of membrane-forming solution was deposited on the hole, the conductivity of the system decreased. The drop thinned gradually and reached a point where the capacitance of the system stopped increasing. This process, depending on the solvent used, took about 5 to 15 min. The plexiglass cell holder and Teflon cup were positioned in a temperature-controlled block. All experiments were carried out at room temperature (21.5 ± 0.5 °C).

Membrane resistance was determined either with a Keithley electrometer (Model 602) or more accurately by applying a current through a pair of Ag-AgCl electrodes (E207 electrodes, In Vivo Metric System, Redwood, Calif.) connected in series with precision high megohm resistances (The Victoreen Instrument Co., Cleveland, Ohio) and a variable voltage source. The membrane potential was measured through another pair of Ag-AgCl electrodes connected to the electrometer and a recorder (Leeds & Northrup, Speedomax XL).

A helium-neon laser (Hughes Aircraft Co., Model 3024 H-P) was used for illumination. The light intensity was measured with a Model 65 radiometer (Yellow Springs Co.) and was 5.5 mW/cm^2 at the level of the membrane. The membrane action spectra were determined with a fiberglass illuminator and light guide (Edmund Scientific Co.) in combination with band pass interference filters (Bausch & Lomb Optical Co. and Baird Atomics) [14]. The total light intensity at the level of the membrane was 230–235 mW/cm^2.

Two different membrane-forming solutions were prepared in decane/*n*-butanol/chloroform mixture (7:2:1 by volume). One consisted of 3 mg/ml L-α-lecithin (from egg yolk, Type III E, Sigma Chemical Co.), 2 mg/ml chlorophyll *a* (from spinach, Sigma) and 1 mg/ml β-carotene (Eastman). The other contained 2.5–3.5 mg/ml of egg lecithin, 1 mg/ml monogalactosyl diglyceride from plant (Supelco, Inc.), 2 mg/ml chlorophyll *a* and 1 mg/ml β-ca-

rotene. To make a homogeneous membrane-forming solution from monogalactosyl diglyceride, a larger amount of chloroform was required so that the amount of chloroform was greater than the amounts of *n*-butanol. Most membrane-forming solutions were used only for one or two days, although at times some solutions exhibited stability for up to five days.

The primary membrane bathing solution was 0.1 M potassium acetate buffer containing 0.5 mM $FeCl_2$ and 0.5 mM $FeCl_3$, pH 5.38. Disodium ethylenediaminetetraacetate (EDTA) was added to a level of 1.5 or 10 mM in an effort to prevent precipitation of ferric hydroxide. After formation of a membrane, $FeCl_3$ was added in a small volume to one side of membrane to increase the concentration to 5.5 mM. Double the amount of $FeCl_3$ was added to solutions containing 10 mM EDTA in order to assure the presence of a photovoltage. To avoid an imbalance of hydrostatic pressure, the same volume of solution was withdrawn from the same side of the membrane. Homogeneity of the membrane bathing solution was attained by stirring with a magnetic microstirring bar. The photovoltage response, the capacitance and the resistance of the system were measured every 10 to 15 min for a half hour. The photovoltage response of a membrane was the difference between the illuminated membrane potential and the dark membrane potential. Once stability in the photoresponse was achieved, a small volume of protein solution (C-phycocyanin or plastocyanin) was added to one side of the membrane. Phycocyanin was always added to the inner compartment of the membrane cell to avoid the absorption of light before reaching the membrane. Plastocyanin could be added to either the inner or outer compartment since the protein does not have an intense absorption band. When the photovoltage response approached a constant value, a second protein was added to the other side or same side of the membrane. Again the photovoltage response, the capacitance and the resistance were recorded.

The potential of the membrane bathing solutions were determined by use of an Orion Model 96–78 combination redox electrode in conjunction with a Beckman Century SS-1 pH meter.

C-Phycocyanin was isolated and purified from the blue-green alga *Plectonema calothricoides* as previously reported [4]. It was stored in a salt-free lyophilized condition. Immediately before the addition to a membrane bathing solution, it was dissolved in 0.1 M potassium acetate buffer, pH 5.38. About 1.0–1.6 mg C-phycocyanin was used in each experiment. The protein concentration was estimated by measuring its absorbance at 620 nm [17]. Its final concentration in the bathing solution was 0.25-0.40 mg/ml.

The fluorescence of C-phycocyanin solutions in the presence and absence of varying concentration of ferric ion was measured as previously reported [13]. The optical density of C-phycocyanin solutions was always 0.2 in a 1-cm cell.

Plastocyanin was extracted and purified from spinach leaves at pH 7 by the method described by Katoh [18]. Before use, it was dialyzed against a large volume of 0.1 M potassium acetate buffer, pH 5.38. The concentration was determined from the absorption difference of its oxidized and reduced forms at 597 nm. In each experiment, 0.2 mg of plastocyanin (in the reduced form) was added to the appropriate side of the membrane by addition to the membrane bathing solution to make a final concentration of 0.0125 mg/ml.

Results

Lecithin Membrane

Bilayer lipid membranes comprised of L-α-lecithin, chlorophyll *a*, and β-carotene were easy to form and were generally stable and photosensitive

for 1 to 3 hr. On occasion membranes were intact for more than 24 hr, but their photosensitivity decreased sharply. The electrical properties were variable from one membrane to another and their capacitances and resistances varied from 2,000 to 5,000 pF and 1.0×10^8 to 1.5×10^9 Ω, respectively. These values are comparable with those of membranes made from chloroplast extract [14].

In a symmetrical system (both sides of the membrane in contact with solutions of the same composition), the membrane dark potential was essentially zero. It decreased substantially when an amount of $FeCl_3$ was added to the side of the membrane connected to a positive lead. The addition of proteins to either side or both sides of membrane did not affect the dark potential. The potential of the membrane-bathing solutions on both sides were regularly monitored and were in the range of 0.35 to 0.40 V depending on the ratio of ferric to ferrous ion.

When a membrane was illuminated, its potential deviated from zero by ± 1 mV even in a symmetrical system. However, zero photopotential was observed if EDTA were present in the bathing solution. It was therefore unlikely that photooxidation of an iron EDTA complex contributed in any way to later observed photopotentials. After the addition of $FeCl_3$ to the membrane-bathing solution on the side that was above ground (positive), a negative photopotential was produced. The subsequent addition of proteins to the appropriate side of the membrane usually caused a further increase in the negative photopotential. The photopotential prior to addition of protein was generally of the magnitude of -2 to -4 mV; after addition of protein the photopotential increased to values as high as -13 mV.

The results of experiments employing C-phycocyanin and plastocyanin are presented in Table 1. The photoenhancements are given as a ratio of the photovoltage response in the presence (ΔV_P) and absence (ΔV_A) of the specific protein. The values reported here are an average of two to six experiments.

In all cases, photoenhancements due to C-phycocyanin developed slower than those of plastocyanin. A photovoltage resulted in minutes after addition of plastocyanin while a lag period of about 15 min was observed with phycocyanin. Published fluorescence measurements [13] have indicated that the maximal interaction of Fe^{3+} with phycocyanin required about 15–30 min. The maximum photoenhancements were generally observed 15 to 30 min after addition of either protein, except in the presence of 10 mM EDTA, where C-phycocyanin did not have an effect. The addition of a second protein to the same membrane induced

Table 1. Effects of phycocyanin and plastocyanin on L-α-lecithin-chlorophyll *a*-β-carotene membranes

	Proteins added to bathing solution	$(\Delta V_P/\Delta V_A)_i$[a]	$(\Delta V_P/\Delta V_A)_{max}\chi$[b]	Concentration of EDTA in bathing solution
I	C-phycocyanin to inside[c]	1.20±0.11 (4)	2.01±0.27 (4)[d]	0
II	C-phycocyanin to inside	1.07±0.09 (6)	1.51±0.44 (6)	1.5 mM
III	C-phycocyanin to inside	0.86	0.86	10.0 mM
IV	Plastocyanin to outside	1.48±0.28 (3)	2.67±0.67 (3)	1.5 mM
V	Plastocyanin to outside	1.93±0.46 (5)	2.04±0.54 (5)	10.0 mM
VI	Plastocyanin to inside	1.0	1.0	0
VII	Plastocyanin to inside	0.91	1.20	10.0 mM
VIII	Plastocyanin to outside	1.48±0.28 (3)	2.67±0.67 (3)	1.5 mM
	then + C-phycocyanin to inside	2.84±0.55 (3)	3.45±1.16 (3)	1.5 mM
IX	Plastocyanin to outside	1.93±0.46 (3)	2.17±0.42 (3)	10.0 mM
	then + C-phycocyanin to inside	1.65±0.55 (3)	2.19	10.0 mM
X	C-phycocyanin to inside	0.99±0.01 (2)	1.67±0.12 (2)	1.5 mM
	then + plastocyanin to outside	2.48±0.53 (2)	3.37±0.63 (2)	1.5 mM
XI	C-phycocyanin to inside	0.86	0.86	10.0 mM
	then + plastocyanin to outside	1.25	1.50	10.0 mM

[a] The ratio of photovoltage responses in the presence, ΔV_P, and absence, ΔV_A, of protein. ΔV_P is measured immediately after addition of protein.

[b] The maximum photovoltage response in the presence of protein was used to calculate the ratio $\Delta V_P/\Delta V_A$.

[c] The side where more oxidant was present.

[d] Number in parenthesis represents number of membranes used.

a further photoenhancement, but the total photoenhancement was smaller than the simple sum of the separate effect of each protein. Reversing the order of addition of the two proteins did not noticeably affect the magnitude of the photoenhancement.

In contrast to C-phycocyanin, plastocyanin had to be added to the side of membrane which was more negative in redox potential (more reductant) in order to have a photoeffect on the BLM. The polarity of the photoresponse was, as in the case with C-phycocyanin, dependent on the redox potential across the membrane and was independent of the direction of illumination. No photoeffect was observed when plasto-

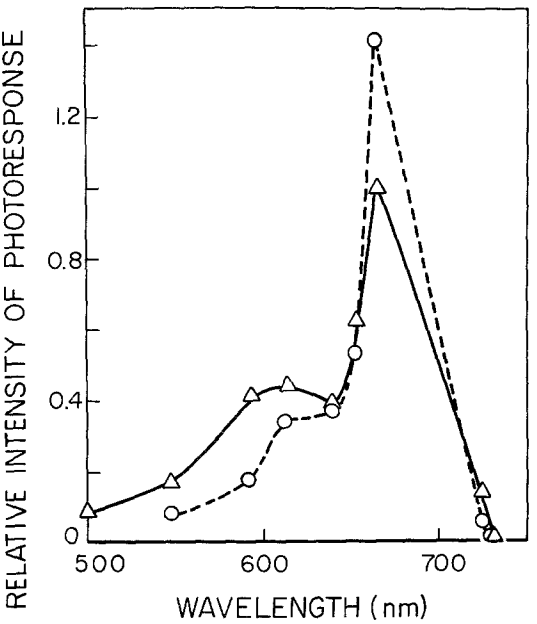

Fig. 1. Typical action spectra of photoeffects with an L-α-lecithin-chlorophyll *a*-β-carotene membrane in the absence and presence of plastocyanin (△—△ and ○----○, respectively). The intensity at each wavelength was determined by dividing the photoresponse ΔV by the relative intensity of the incident light. In order to present both spectra on the same scale, the photoresponse intensity of the membrane in the absence of plastocyanin at 665 nm was set arbitrarily as 1.0. Membrane bathing solution inside, 0.5 mM $FeCl_3$ + 0.5 mM $FeCl_2$ + 10 mM EDTA + 0.1 M K-acetate, pH 5.35; outside, 20.5 mM $FeCl_3$ + 0.5 mM $FeCl_2$ + 10.0 mM EDTA + 0.1 K-acetate. Plastocyanin was added to the inner compartment

cyanin was added to the more oxidized side of membrane. However, since the addition of plastocyanin to that side of the membrane produced some precipitation, the absence of a photoenhancement effect was not necessarily meaningful. In Fig. 1, the action spectra of the membrane photoeffects in the presence and absence of plastocyanin were similar. Each spectrum had a peak at 665 nm and a shoulder at 620 nm. The one with plastocyanin had a higher intensity around 665 nm compared to the other (41% higher). These action spectra were comparable to the long wavelength portion of the chlorophyll absorption spectra [9].

Monogalactosyl Diglyceride-Lecithin Membrane

Since monogalactosyl diglyceride is a major component of chloroplast extract and thylakoid membranes [1, 7], attempts were made to use this

lipid as the major component in the membrane-forming solution; however, no stable membranes were obtained. Many attempts were also made to produce membranes from different mixtures of monogalactosyl diglyceride and L-α-lecithin. It was found that stable membranes could be formed if the concentrations of L-α-lecithin and monogalactosyl diglyceride in the forming solution were 2.5–3.5 mg/ml and 1 mg/ml, respectively. These membranes were not easy to produce, but after formation they were photosensitive and very stable. Most of them were not only intact for more than 15 hr, but also retained their photosensitivity. One membrane was made which lasted more than 14 days. As in L-α-lecithin membrane, the electrical properties varied from one membrane to the other. Membrane capacitances and resistances were in the range of 1,500 to 5,500 pF and 1.0×10^8 to 2.0×10^9 Ω, respectively. Membrane capacitance and resistance were essentially constant throughout the experiment.

When proteins were added to appropriate sides of the membrane in sequence, the photoresponse was modified correspondingly. The photoenhancement due to the C-phycocyanin and plastocyanin are presented in Table 2. The photovoltage prior to addition of protein was −2 to −4 mV and after addition of protein the photovoltage increased to values as high as −36 mV. The photoenhancements increased slowly and approached maximum values (10 to 120 min after the addition of the first

Table 2. Effects of phycocyanin and plastocyanin on L-α-lecithin-monogalactosyl diglyceride-chlorophyll *a*-β-carotene membranes

	Proteins added to bathing solution[a]	$(\Delta V_P/\Delta V_A)_i$	$(\Delta V_P/\Delta V_A)_{max}$	t[b]	Membranes' lifetime
I	C-phycocyanin to inside then + plastocyanin to outside	1.22 ± 0.18 (4) 2.10 ± 0.91 (4)	1.90 ± 0.68 (4) 2.62 ± 1.20 (4)	49 ± 16 min 32 ± 17 min	> 20 hr
II	Plastocyanin to outside then + C-phycocyanin to inside	1.45 ± 0.38 (4) 4.08 ± 1.48 (4)	3.86 ± 1.26 (4) 5.16 ± 0.67 (4)	1 hr 57 ± 27 min 19 ± 2 hr	> 15 hr
IIa	Plastocyanin to outside then + C-phycocyanin to inside	1.0 6.67	1.67 17.11	42 min 21 hr 14 min	> 70 hr
IIb	Plastocyanin to outside then + C-phycocyanin to inside	2.65 7.25	9.25 18.25	4 hr 45 min 18 hr 18 min	> 14 days

[a] All membrane bathing solutions employed here contained 1.5 mM EDTA.

[b] Time lapse between addition of protein and observation of maximum photovoltage response. Other notations are the same as in Table 1.

protein; namely, plastocyanin or C-phycocyanin). When the second protein added was phycocyanin, the maximum photoeffect was observed 17 to 21 hr after addition while in experiments with plastocyanin as the second protein the maximum photoeffect occurred 15 to 30 min after addition. The total effect of the proteins on the membrane depended on the order of protein addition to the bathing solution. The addition of plastocyanin to the membrane prior to phycocyanin always resulted in a greater total photoenhancement and it was necessary to add plastocyanin to the more reduced side of the membrane and C-phycocyanin to the opposite side.

The action spectrum of photoeffects of monogalactosyl-lecithin membrane in the absence of protein was similar to the long wavelength portion of chlorophyll absorption spectrum. In the presence of both

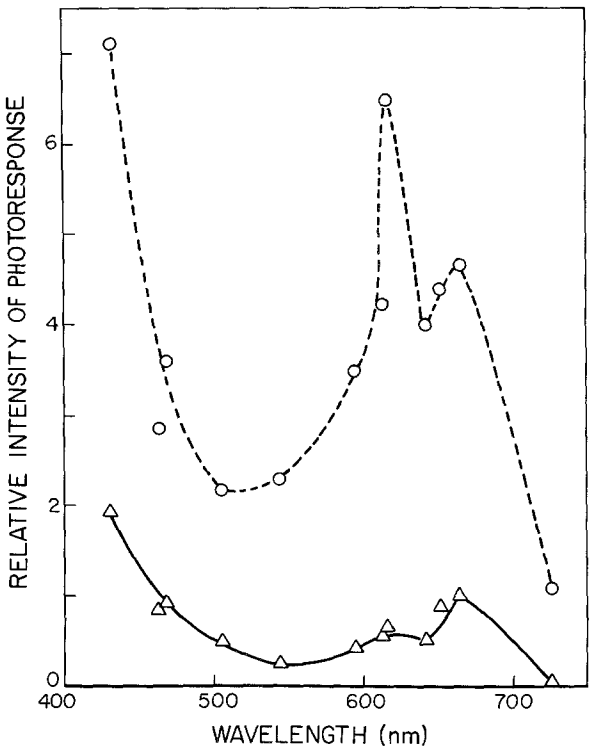

Fig. 2. Typical action spectra of photoeffects with an L-α-lecithin-monogalactosyl diglyceride-chlorophyll *a*-*β*-carotene membrane in the absence and presence of both C-phycocyanin and plastocyanin (△—△ and ○----○, respectively). Membrane-bathing solution inside, 5.5 mM $FeCl_3$+0.5 mM $FeCl_2$+1.5 mM EDTA+0.1 M K-acetate; outside, 0.5 mM $FeCl_3$+0.5 mM $FeCl_2$+1.5 mM EDTA+0.1 M K-acetate, pH 5.35. Plastocyanin was added to the outside and C-phycocyanin was added to the inside solution. The intensity at each wavelength was determined as in Fig. 1

C-phycocyanin and plastocyanin, the action spectrum of photoeffects (Fig. 2) had peaks around 616 and 665 nm at long wavelength region. The peak around 616 nm had relatively higher intensity (15 to 40% higher compared to that at 665 nm) and was coincident with the absorption peak of C-phycocyanin.

Discussion

Upon irradiation, C-phycocyanin, located on the more oxidized side of L-α-lecithin-chlorophyll *a*-β-carotene membrane, is able to facilitate electron transport across the membrane as well as it does in BLM made from chloroplast extract [6, 14, 19]. This ability is reduced and eventually inhibited by increasing the concentration of EDTA in the bathing solution. The observations suggest that (i) the photoaction of C-phycocyanin on BLM occurs with each type of lipid used in the membrane; (ii) ferric ions are required so that they may form a complex with phycocyanin [13], chelating the ferric ion with excess EDTA attenuates the photoenhancement; (iii) the interaction between C-phycocyanin and the membrane occurs at the membrane-aqueous interface and not in the lipophilic portion of the membrane. Therefore, the function of C-phycocyanin is, as proposed in a previous paper [15], to reduce the energy barrier for electron transport across the membrane-aqueous interface. The reduction of this energy barrier was accomplished through the formation of phycocyanin-Fe^{3+} complex at the interface [13]. The exact stoichiometry of the Fe^{3+} phycocyanin interaction is at present experimentally difficult to obtain. Fluorescence quenching experiments at constant protein concentration, while varying Fe^{3+} concentration, indicate that at a protein concentration of 1×10^{-6} M and Fe^{3+} concentration of $\sim 5 \times 10^{-5}$ there is substantial fluorescence quenching. The molar concentration for phycocyanin is calculated on the basis of a monomer of about 30,000 daltons [16]. Each monomer unit probably has 3 tetrapyrrole chromophores [26]; therefore there are 3 chromophores or binding sites for ferric ion per monomer. If we assume that an equilibrium constant of the order of 10^4 exists for the binding of a single ferric ion to the biliprotein monomer, it is clearly possible that a one-to-one stoichiometry of the ferric ion to phycocyanin chromophore exists. The observation that EDTA attenuates the ability of C-phycocyanin to facilitate electron transport across the membrane requires that the preliminary results reported by Mangel [19] be considered with caution since EDTA was

present in those experiments. It is premature to suggest a detailed mechanism for the effect of EDTA in these experiments; however, it does seem clear that chelation of ferric ion inhibits the effects of phycocyanin and in the absence of EDTA and in the presence of ferric ion there is a tendency for plastocyanin to precipitate.

Experimentally one of the most difficult situations to deal with is the use of the ferric, ferrous ion redox couple. The ferric ion is most difficult to maintain in solution due to its tendency to form aquo-hydroxy complexes [8]. It is for this reason that we choose to work in 0.1 M potassium acetate buffer. This usually represents a 100-fold excess of acetate ion which does have a good tendency to complex with the ferric ion [8] to the extent that it retards formation of the aquo-hydroxy complexes. The difficulty in utilizing other metal ion redox pairs for work with the biliprotein rests at this point with the observation that ferric ion biliprotein interaction is apparently specific [13] and not shared with any of the common metal ions investigated. It is important to state that there is no extended time-dependent effect of the photovoltage which can be correlated with possible changes in the state of the ferric ion over periods of many hours. Manipulation of the difference in redox potential on opposite sides of the membrane can and has been accomplished using ascorbic acid and cerous, ceric ion redox pairs [14]; however, for the experiments employing biliproteins the presence of ferric ion has proven essential.

Plastocyanin is a copper protein known as an electron carrier in photosynthetic systems [2, 18]. When it is added to the more reduced side of membrane, it modifies the photoresponse in a manner analogous to C-phycocyanin except that this protein is operating on the side of the membrane in which electrons are injected into the membrane. On the other hand, the presence of plastocyanin on the more oxidized side of the membrane causes no significant change in photoresponse. The photoactions are independent of the direction of illumination. These experimental results imply that plastocyanin, in contrast to C-phycocyanin, facilitates electron transport from aqueous solution to the membrane, but not vice versa. Although there is no intention to imply a direct correlation with the photosynthetic process, it is of interest to examine the role of plastocyanin in the biological system. The electron transport scheme associated with Photosystem II is currently visualized in the following manner [2]: cytochrome *b* 559 → plastoquinone → cytochrome *f* → plastocyanin → P700. Therefore it would be predicted that plastocyanin would be effective on the side of the membrane where it may assist

electron transfer to the chlorophyll in the membrane. The mechanism of action is probably through the reduction of the energy barrier for electron movement across the interface at the more reduced side of membrane. It is also probable that the copper ion in the protein and the specific structure of the protein may assist in the electron tunneling process [15]. A simple consideration of the redox potential of plastocyanin [18] ($E_o = +0.37$ V) and the measured redox potential of the ferric, ferrous ion couple in the presence of acetate and EDTA as used in these experiments ($\sim +0.35$ V) would indicate that it is indeed possible for the plastocyanin to be continually reduced by the ferrous, ferric ion couple. The interaction of the plastocyanin with the membrane would certainly serve to modify the potential difference and the presence of EDTA and acetate ion presents the possibility of several different types of ferric ion species being available for interaction with the plastocyanin.

It is premature to suggest a specific mechanism to explain the facilitated electron transfer that is stimulated by the presence of C-phycocyanin and plastocyanin on either side of the membrane, but it is possible to indicate some likely characteristics of the mechanism. Both proteins may reduce the barrier to electron transport between the Fe^{3+}/Fe^{2+} aqueous phase and the chlorophyll a/β-carotene lipid phase by making the coupling between the two phases more efficient. C-phycocyanin is adsorbed at the interface and complexes Fe^{3+} ions, thereby bringing the oxidizing agent (Fe^{3+}) closer to the pigments. The Fe^{3+} ion complexed presumably with the C-phycocyanin chromophore could result in some π bond complexing, and the delocalization of the electron could lead to overlap between an electron in the chlorophyll moiety and the Fe^{3+}-C-phycocyanin complex which would facilitate the electron transfer. Plastocyanin (a copper-containing protein) is added in its reduced form and is adsorbed at the interface, thereby bringing the reducing agent closer to the pigments in the membrane. Redox equilibria exist between the Fe^{3+}/Fe^{2+} (EDTA) pool in the aqueous phase and both proteins. The plastocyanin copper ion complex could also be involved in π electron delocalization and overlap with the chlorophyll moiety which facilitates electron transfer to the chlorophyll.

It seems that the metal ions in the two proteins studied play an important role in assisting electron transport across the membrane interface. C-Phycocyanin which when purified contains no metal ion has to react with ferric ion and presumably forms a complex before it can facilitate electron transport [13], while plastocyanin, which already has a copper ion, may act as soon as it is added to the membrane-bathing

solution. It is possible that the time lag observed with C-phycocyanin is related to the ferric ion interaction characterized previously by a time-dependent fluorescence quenching [13]. These arguments are supported by the experimental observations: (i) C-phycocyanin is only capable of modifying the photoresponse in the presence of ferric ion that is not completely complexed by EDTA; (ii) plastocyanin modifies the photoresponse as soon as it is added to aqueous solution adjacent to membrane; (iii) in general, a 10 to 15 min time lapse between the addition of C-phycocyanin to bathing solution and modification of the photoresponse is required.

The lack of a cooperative effect of C-phycocyanin and plastocyanin with L-α-lecithin membrane indicates that upon illumination each protein works independently at each membrane-aqueous interface. The proteins are found to act cooperatively on the membrane containing monogalactosyl diglyceride. It is possible that monogalactosyl diglyceride molecules provide a favorable condition for the two proteins to work synergistically. However, since reversing of the order of protein additions to the membrane-bathing solution produces no cooperative effect, the existence of monogalactosyl diglyceride in the membrane separating the two proteins is not a sufficient condition for the realization of the synergy. If it is assumed that the action of a protein is a result of its interaction with an appropriate membrane-aqueous interface, the reaction of one protein with a membrane surface may change the membrane's structure which may or may not be suitable for the reaction of the second protein at the other side of the membrane.

The unusual stability and high photoefficiency of the artifical membranes containing monogalactosyl diglyceride (compared to the L-α-lecithin membranes) and the high concentration of this lipid in thylakoid membranes suggest that this lipid may play an important role in the photosynthetic system. It is tempting to suggest that the molecular structure and properties of monogalactosyl diglyceride provides favorable elements for building a specific membrane architecture that can withstand environmental stress and possibly facilitates the aggregation of chlorophyll *a*. According to the theoretical model suggested previously [15], assemblies of chlorophyll molecules are required to enhance the process of electron transport across the membrane.

The data presented in Table 2 (Experiment *II, IIa, IIb*) shows that under the same experimental conditions, the effects of C-phycocyanin and plastocyanin vary widely. This is possibly due to different local concentrations and an uneven distribution of monogalactosyl diglyceride

in each membrane. Since monogalactosyl diglyceride is not soluble in decane and *n*-butanol, it is initially mixed with other constituents of the membrane-forming solution by adding a small amount of chloroform. When the forming solution is applied to the aperture in a Teflon cup, the chloroform recedes from the membrane, then evaporates or dissolves in the aqueous solution. During this time monogalactosyl diglyceride may stay and distribute among other molecules or escape from the membrane. Consequently its concentration and distribution in each membrane is expected to be different although the same membrane-forming solution is employed. It is anticipated that monolayer studies of the lipids, chlorophyll, β-carotene, and protein will be capable of contributing valuable information about the packing arrangement of the several molecules and their mutual interaction. This in turn could lead to new possibilities for construction of BLM with higher quantum efficiency and greater stability.

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